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     FILE 'MEDLINE' ENTERED AT 14:39:38 ON 01 FEB 2005
L1          237 S (APO-2 LIGAND) OR (APO2 LIGAND) OR (APO-2L) OR (APO2L)
L2         3428 S L1 OR TRAIL
L3         1455 S L2 AND PY<2000
L4            8 S L3 AND ZINC
L5            2 S L3 AND (TRIMER OR TRIMERIZED OR TRIMERS OR TRIMERIZATION)

     FILE 'STNGUIDE' ENTERED AT 14:43:06 ON 01 FEB 2005
L6            0 S L2 AND ZINC NOT POISON?
L7            0 S L2 AND ZINC
L8            0 S L1 AND ZINC

     FILE 'MEDLINE' ENTERED AT 14:49:18 ON 01 FEB 2005
L9           19 S L2 AND ZINC

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L9 ANSWER 1 OF 19 MEDLINE on STN
TI Specificity of molecular recognition learned from the crystal structures of **TRAIL** and the **TRAIL**:sDR5 complex.
AU Cha Sun-Shin; Song Young-Lan; Oh Byung-Ha
SO Vitamins and hormones, (2004) 67 1-17. Ref: 58
Journal code: 0413601. ISSN: 0083-6729.

L9 ANSWER 2 OF 19 MEDLINE on STN
TI Modulation of death receptor pathways in oncology.
AU de Vries E G E; Timmer T; Mulder N H; van Geelen C M M; van der Graaf W T A; Spierings D C J; de Hooge M N; Gietema J A; de Jong S
SO Drugs of today (Barcelona, Spain : 1998), (2003) 39 Suppl C 95-109. Ref: 87
Journal code: 101160518. ISSN: 0025-7656.

L9 ANSWER 4 OF 19 MEDLINE on STN
TI Identification of X-linked inhibitor of apoptosis-associated factor-1 as an interferon-stimulated gene that augments **TRAIL Apo2L** -induced apoptosis.
AU Leaman Douglas W; Chawla-Sarkar Mamta; Vyas Keyur; Reheman Monila; Tamai Katsuyuki; Toji Singo; Borden Ernest C
SO Journal of biological chemistry, (2002 Aug 9) 277 (32) 28504-11.
Journal code: 2985121R. ISSN: 0021-9258.

L9 ANSWER 9 OF 19 MEDLINE on STN
TI Functional analysis of tumour necrosis factor-alpha-related apoptosis-inducing ligand (**TRAIL**): cysteine-230 plays a critical role in the homotrimerization and biological activity of this novel tumoricidal cytokine.
AU Trabzuni D; Famulski K S; Ahmad M
SO Biochemical journal, (2000 Sep 1) 350 Pt 2 505-10.
Journal code: 2984726R. ISSN: 0264-6021.

L9 ANSWER 10 OF 19 MEDLINE on STN
TI Cysteine 230 is essential for the structure and activity of the cytotoxic ligand **TRAIL**.
AU Bodmer J L; Meier P; Tschopp J; Schneider P
SO Journal of biological chemistry, (2000 Jul 7) 275 (27) 20632-7.
Journal code: 2985121R. ISSN: 0021-9258.

L9 ANSWER 11 OF 19 MEDLINE on STN
TI A unique **zinc**-binding site revealed by a high-resolution X-ray structure of homotrimeric **Apo2L/TRAIL**.
AU Hymowitz S G; O'Connell M P; Ultsch M H; Hurst A; Totpal K; Ashkenazi A; de Vos A M; Kelley R F
SO Biochemistry, (2000 Feb 1) 39 (4) 633-40.
Journal code: 0370623. ISSN: 0006-2960.

FILE 'HOME' ENTERED AT 14:38:29 ON 01 FEB 2005

FILE 'MEDLINE' ENTERED AT 14:39:38 ON 01 FEB 2005

L1 237 S (APO-2 LIGAND) OR (APO2 LIGAND) OR (APO-2L) OR (APO2L)
L2 3428 S L1 OR TRAIL
L3 1455 S L2 AND PY<2000
L4 8 S L3 AND ZINC
L5 2 S L3 AND (TRIMER OR TRIMERIZED OR TRIMERS OR TRIMERIZATION)

FILE 'STNGUIDE' ENTERED AT 14:43:06 ON 01 FEB 2005

L5 ANSWER 1 OF 2 MEDLINE on STN
SO Nature medicine, **(1999 Feb)** 5 (2) 157-63.
Journal code: 9502015. ISSN: 1078-8956.
AB To evaluate the utility of tumor necrosis factor-related apoptosis-inducing ligand (**TRAIL**) as a cancer therapeutic, we created leucine zipper (LZ) forms of human (hu) and murine (mu) **TRAIL** to promote and stabilize the formation of **trimers**. Both were biologically active, inducing apoptosis of both human and murine target cells in vitro with similar specific activities. In contrast to the fulminant hepatotoxicity of LZ-huCD95L in vivo, administration of either LZ-huTRAIL or LZ-muTRAIL did not seem toxic to normal tissues of mice. Finally, repeated treatments with LZ-huTRAIL actively suppressed growth of the **TRAIL**-sensitive human mammary adenocarcinoma cell line MDA-231 in CB.17 (SCID) mice, and histologic examination of tumors from SCID mice treated with LZ-huTRAIL demonstrated clear areas of apoptotic necrosis within 9-12 hours of injection.

L5 ANSWER 2 OF 2 MEDLINE on STN
SO Journal of experimental medicine, **(1998 Apr 20)** 187 (8) 1205-13.
Journal code: 2985109R. ISSN: 0022-1007.
AB Human Fas ligand (L) (CD95L) and tumor necrosis factor (TNF)-alpha undergo metalloproteinase-mediated proteolytic processing in their extracellular domains resulting in the release of soluble trimeric ligands (soluble [s]FasL, sTNF-alpha) which, in the case of sFasL, is thought to be implicated in diseases such as hepatitis and AIDS. Here we show that the processing of sFasL occurs between Ser126 and Leu127. The apoptotic-inducing capacity of naturally processed sFasL was reduced by >1,000-fold compared with membrane-bound FasL, and injection of high doses of recombinant sFasL in mice did not induce liver failure. However, soluble FasL retained its capacity to interact with Fas, and restoration of its cytotoxic activity was achieved both in vitro and in vivo with the addition of cross-linking antibodies. Similarly, the marginal apoptotic activity of recombinant soluble TNF-related apoptosis-inducing ligand (sTRAIL), another member of the TNF ligand family, was greatly increased upon cross-linking. These results indicate that the mere **trimerization** of the Fas and **TRAIL** receptors may not be sufficient to trigger death signals. Thus, the observation that sFasL is less cytotoxic than membrane-bound FasL may explain why in certain types of cancer, systemic tissue damage is not detected, even though the levels of circulating sFasL are high.

=> d ti, au, so 1-2

L5 ANSWER 1 OF 2 MEDLINE on STN
TI Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo.
AU Walczak H; Miller R E; Ariail K; Gliniak B; Griffith T S; Kubin M; Chin W; Jones J; Woodward A; Le T; Smith C; Smolak P; Goodwin R G; Rauch C T; Schuh J C; Lynch D H
SO Nature medicine, **(1999 Feb)** 5 (2) 157-63.
Journal code: 9502015. ISSN: 1078-8956.

L5 ANSWER 2 OF 2 MEDLINE on STN
TI Conversion of membrane-bound Fas(CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity.
AU Schneider P; Holler N; Bodmer J L; Hahne M; Frei K; Fontana A; Tschopp J
SO Journal of experimental medicine, **(1998 Apr 20)** 187 (8) 1205-13.
Journal code: 2985109R. ISSN: 0022-1007.

et al., 1993) or the pBluescript SK(+) (Stratagene) vector to create pDC409-TRAIL and pBluescript-TRAIL, respectively.

pDC409-Flag-TRAIL was created by PCR amplification of TRAIL cDNA encoding amino acids 95–281. The 3′ oligonucleotide was the same as used to create pDC409-TRAIL and the 5′ oligonucleotide was GTCACTAGTTCTGACTACAAGGACGACGATGACAAGACCTCTGAGGAAACCATTTC, which adds a SpeI site and synthetic Flag epitope encoding region to the 5′ end (Hopp et al., 1988). The resulting PCR product was cut with SpeI and NotI, and inserted into SalI and NotI cut pDC409 along with annealed oligonucleotides encoding a putative CMV open reading frame leader (Rawlinson and Barrell, 1993). This produced an open reading frame encoding the CMV leader, the Flag epitope, and human TRAIL amino acids 95–281. A parallel construct, pDC409-Flag-muTRAIL, was created in the same way but using a PCR fragment generated from the murine TRAIL cDNA using the 5′ and 3′ PCR primers GCGTCACTAGTTCTGACTACAAGGACGACGATGACAAGACCTTTCAGGACACCATTTC and ATAGCGGCCGCTGTGTTTGATCTTTACTGGTC, respectively.

Purification of Soluble TRAIL

Supernatants from CV1/EBNA cells were harvested 3 days after transfection with pDC409-Flag-TRAIL. These were applied to a column containing the M2 anti-Flag antibody (Hopp et al., 1988), immobilized to a solid support, and washed with PBS. Fractions (800 ml) were eluted with 50 mM citrate and immediately neutralized in 0.45 ml 1M Tris (pH 8). Fractions were adjusted to 10% glycerol and stored at −20°C until needed.

RNA Hybridization

Northern blot analysis of RNA samples was performed by using Clonetech (Palo Alto, California) multiple tissue Northern blots I and II, or by resolving RNA samples on a 1.1% agarose-formaldehyde gel and blotting onto Hybond-N as recommended by the manufacturer (Amersham Corporation), and staining with methylene blue to monitor RNA concentrations. Antisense RNA probe was generated using T3 RNA polymerase and pBluescript-TRAIL linearized with SalI as template.

DNA Laddering Apoptosis Assay

CV1/EBNA cells grown in Falcon T175 flasks were transfected with 15 μg of either pDC409 or pDC409-TRAIL vector. These cells were then cultured for 3 days at 37°C and 10% CO_2, then fixed as previously described (Smith et al., 1993). Of these cells, 4×10^4 per well were cocultured in a 6-well plate with 2.5 ml of medium with the indicated combinations of fixed cells or concentrated supernatants from COS cells transfected with Fas ligand. Indicated cells were pretreated for 10 min with 10 μg/ml of M3, a monoclonal antibody to Fas receptor (Ramsdell et al., 1994; Alderson et al., 1995). After incubation for 4 hr at 37°C and 10% CO_2, fragmented DNA in the cytoplasm was recovered as described (Ishida et al., 1992), except the cell lysates were extracted three times with 1 ml of 25:24:1 phenol–chloroform–isoamyl alcohol, and ethanol precipitated in the presence of 5 μg of glycogen carrier

Percent Viability Assay

Cells were incubated with the indicated factors in 96-well plates in a volume of 100 μl, and assayed by alamar blue (Figure 5A; Table 1) or crystal violet (Figure 6). All cells were cultured at 5×10^3 cells per well except for A375 cells, which were cultured at 1×10^4 cells per well. Where indicated, immobilized anti-Flag antibody, M2, was added at a concentration of 10 μg/ml in a volume of 100 μl per well (Hopp et al., 1988) and allowed to adhere either overnight at 4°C or 2 hr at 37°C, then aspirated and washed twice with PBS. The incubation period was 20 hr for all cells, except the A375 cells, which were incubated for 72 hr. Conditioned supernatants were used at a concentration of 10 μl per well. Alamar blue conversion was measured by adding 10 μl of alamar blue dye (Biosource International, Camarillo, California) per well, and subtracting the OD at 550–600 nm at the time the dye was added from the OD 550–600 nm after 4 hr. No conversion of dye is plotted as 0% viability, and the level of dye conversion in the absence of TRAIL is plotted as 100% viability. When shown, error bars represent the standard deviation of measurements from four independent wells, and the values are the average of these measurements. Crystal violet staining was performed as described (Flick and Gifford, 1984). Percent viability is calculated by multiplying the ratio staining of experimental versus control cultures by 100. To confirm that the changes in dye activities of both the alamar blue and crystal violet assay were due to cell death, the decrease in cell viability induced by TRAIL was confirmed by staining the cells with trypan blue

Confocal Microscopy

Live/dead viability/cytotoxicity assays were performed as recommended by the manufacturer (Molecular Probes, Incorporated, Eugene, Oregon). BODIPY FL phallacidin and propidium iodide were purchased from Molecular Probes. Cells were stained with these reagents and visualized using a confocal laser scanning microscope as recommended by the microscope manufacturer (Molecular Dynamics, Sunnyvale, California).

Chromosomal Mapping

The human TRAIL coding region was nick-translated with biotin-14-dATP and hybridized in situ at a final concentration of 20 ng/ml to metaphases from normal males. The fluorescence in situ hybridization method was modified from that previously described (Callen et al., 1990), in that chromosomes were stained before analysis with both propidium iodide (as counterstain) and DAPI (for chromosome identification). Images of metaphase preparations were captured by CCD camera and computer enhanced.

Acknowledgments

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References

Adachi, M., Watanabe-Fukunaga, R., and Nagata, S. (1993). Aberrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of *lpr* mice. Proc. Natl. Acad. Sci. USA *90*, 1756–1760.

Alderson, M.R., Tough, T.W., Davis-Smith, T., Braddy, S., Falk, B., Schooley, K.A., Goodwin, R.G., Smith, C.A., Ramsdell, F., and Lynch, D.H. (1995). Fas ligand mediates activation-induced cell death in human T lymphocytes. J. Exp. Med. *181*, 71–77.

Allen, R.C., Armitage, R.J., Conley, M.E., Rosenblatt, H., Jenkins, N.A., Copeland, N.G., Bedell, M.A., Edelhoff, S., Disteche, C.M., Simoneaux, D.K., Fanslow, W.C., Belmont, J., and Spriggs, M.K. (1993). CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science *259*, 990–993.

Browning, J.L., Ngam-ek, A., Lawton, P., DeMarinis, J., Tizard, R., Chow, E.P., Hession, C., O'Brien-Greco, B., Foley, S.F., and Ware, C.F. (1993). Lymphotoxin β, a novel member of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface. Cell *72*, 847–856.

Callen, D.F., Baker, E., Eyre, H.J., Chernos, J.E., Bell, J.A., and Sutherland, G.R. (1990). Reassessment of two apparent deletions of chromosome 16p to an ins(11;16) and a t(1;16) by chromosome painting. Ann. Genet. *33*, 219–221.

Cayabyab, M., Phillips, J.H., and Lanier, L.L. (1994). CD40 preferentially costimulates activation of CD4+ T lymphocytes. J. Immunol. *152*, 1523–1531.

Cerami, A., and Beutler, B. (1988). The role of cachectin/TNF in endotoxic shock and cachexia. Immunol. Today *9*, 28–31.